

### **ABSTRACT OF THE DISCLOSURE**

The invention relates to methods and systems for predicting or estimating the melting temperature of duplex nucleic acids, particularly duplexes of oligonucleotides which may be used, for example, as primers or probes in PCR and/or hybridization assays. The invention also relates to methods and systems for designing and selecting oligonucleotide probes and primers having a predicted melting temperature which is optimized for such assays. To this end, algorithms and methods are provided for predicting the melting temperature of a nucleic acid having a predetermined sequence. These methods and algorithms estimate the melting temperature of a nucleic acid duplex under particular salt conditions. The methods and algorithms use novel formulas, having terms and coefficients that are functions of the particular nucleotide sequence, to estimate the effect of particular salt conditions on the melting temperature. As such, the methods and systems of the invention provide superior result compared to existing methods, which do not consider sequence dependent effects of changing salt conditions.